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(71) Applicant: CELL THERAPEUTICS, INC. [US/US]; Suite 400, 201 Elliott Avenue West, Seattle, WA 98119 (US).

(72) Inventors: TODARO, George, J.; 1940 Fifteenth Avenue East, Seattle, WA 98112 (US). LEUNG, David, W.; 7625 East Mercer Way, Mercer Island, WA 98040 (US). ROSE, Timothy, M.; 5045 N.E. 70th Street, Seattle, WA 98115 (US).

(74) Agent: FACISZEWSKI, Stephen; Cell Therapeutics, Inc., Suite 400, 201 Elliott Avenue West, Seattle, WA 98119 (US).

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(54) Title: HYBRID CYTOKINES

(57) Abstract

Therapeutic hybrid cytokines, having a size ranging from about 10 to about 30 kDa, comprise portions of cytokines: leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF), interleukin-6 (IL-6), interleukin-11 (IL-11), oncostatin-M (OSM), and ciliaryneurotrophic factor (CNTF). Hybrid cytokines comprise three or four α-helical sequences selected from α-helical sequences of IL-6, G-CSF, LIF, IL-11, CNTF and OSM and linking sequences of 5-40 amino acids in length, selected from the linking sequences of IL-6, G-CSF, LIF, IL-11, CNTF and OSM or other desirable linking sequences. In the hybrid cytokines, at least one α-helical sequence is derived from a different cytokine than at least one other α-helical sequence; or, at least one linking sequence of a cytokine differentiates the hybrid cytokine from a corresponding cytokine. In hybrid cytokines having three α-helical sequences selected from the group of cytokines listed, a possible fourth sequence may not correspond to any α-helical sequence in any of the cytokines. Hybrid cytokines, radically different from any corresponding cytokines, have unexpected advantages in selective biological mechanisms over a much broader range of biological activities than exhibited by cytokines.

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HYBRID CYTOKINES

Technical Field

The invention concerns hybrid cytokines which have beneficial, therapeutic properties and comprise amino acid sequence components from cytokine family members leukemia inhibitory factor (LIF); granulocyte colony stimulating factor (G-CSF); interleukin-6 (IL-6); interleukin-11 (IL-11); ciliary neurotrophic factor (CNTF); and oncostatin-M (OSM).

Background of the Invention

More than two dozen cytokines have been identified that regulate blood composition by controlling the growth and differentiation of hematopoietic stem cells. Interferons, tumor necrosis factor, stem cell factor, the numbered interleukins, ligands of oncogene receptors, and the various colony stimulating factors are exemplary of these proteins and glycoproteins. One of these factors, interleukin-6 (IL-6) was originally identified as a B-cell differentiation factor, but has subsequently been shown to induce acute phase protein synthesis in liver cells, to inhibit growth of certain myeloid leukemia cell lines and induce their differentiation into macrophage cells, to promote IL-3 dependent colony formation of primitive blast colony forming cells, to cause differentiation of neuronal cells, to enhance keratinocyte and mesangial cell growth, to promote the maturation of megakaryocytes, and to induce the proliferation and differentiation of T cells. *In vivo*, IL-6 increases the hematopoietic cell count of the erythroid, myeloid, and thrombocytic lineages. Other former names for IL-6 are β2-interferon, B-cell stimulatory factor-2, hybridoma/plasmacytoma growth factor, and monocyte granulocyte inducer type 2. The spectrum of activities attributable to IL-6 indicates that it is useful in tumor inhibition, bone remodeling, kidney development, and T- and B-cell proliferation and stimulation.

Interleukin-11 has been shown to augment hematopoietic proliferation and differentiation of cells from normal mice, increase B-cell maturation, augment macrphage proliferation and megakaryocyte maturation, proliferate multipotent hematopoietic progenitors, stimulate early murine progenitors, and inhibit adipogenesis. Burstein *et al.*, Journal of Cellular Physiology (1992) 153:312; Bazan, Neuron (1991) 7:197; and Yang *et al.*, BioFactors (1992) 4:15-21.

Ciliary neurotrophic factor (CNTF) receptor is most homologous to the IL-6 receptor and lacks a cytoplasmic domain. Except for skeletal muscle, peripheral (sciatic) nerve and adrenal gland, CNTF receptor expression appears confined to the central nervous system. CNTF has been shown to promote neuronal differentiation and neuron augmentation. Research results implicate CNTF in the trophic support of a broad range of peripheral and central neurons, broader in fact, than that of the neurotrophins. Lo, Proc. Natl. Acad. Sci. USA (1993) 90; 2557-2558. Specifically, CNTF has shown "rescue" effects on embryonic spinal cord motor neurons, axotomized facial motor neurons in young rats and degenerative motor neurons in mouse mutant progressive motor neuropathy. *In vivo*, CNTF infused into the lateral ventrical

of fimbria-fornix-lesioned adult rats prevents degeneration of almost all septal neurons including many non-cholinergic neurons that are not maintained by nerve growth factor. Unsicker *et al.*, Neurobiology (1992) 2:671-678.

Leukemia inhibitory factor (LIF) has been demonstrated to inhibit the growth of certain myeloid leukemia cells and to induce their differentiation into macrophage cells; to enhance interleukin-3 dependent colony formation of primitive blast cells; to promote megakaryocyte growth and differentiation; to induce neuronal differentiation; to stimulate the production of acute phase proteins and hepatocytes (all properties it shares with IL-6) and to inhibit the differentiation of embryonic stem cells and kidney cells and to induce bone resorption.

Oncostatin-M (OSM) is known to be a tumor inhibitor for melanoma and certain carcinoma cells and inhibits the growth of human A375 melanoma cells but not normal human fibroblasts. It is also an inhibitor of the growth of M1 myeloid leukemic cells and induces their differentiation into macrophage-like cells as well as stimulating megakaryocyte production in the spleen. OSM is also known to inhibit embryonic stem cell differentiation, induce hepatic cell acute-phase protein sythesis, induce mitosis of AIDS-related Kaposi's sarcoma cell and vascular smooth muscle cell and induce neuronal differentiation.

Granulocyte colony stimulating factor (G-CSF) stimulates neutrophil proliferation and differentiation and induces the differentiation of M1 murine myeloid leukemic cells into macrophage-like cells as well as enhancing interleukin-3 dependent colony formation of primitive blast cells. It appears to have little effect on the hematopoietic cell lineages of megakaryocytes or platelets but enhances cytosine arabinoside-mediated cytoxicity in human myeloid leukemia cells.

Reported biological activities of the foregoing cytokine family members are summarized in the following table:

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TABLE I

Reported Biological Activities of Cytokine Family Members

	LIF	OSM	G-CSF	IL-6	L-11	CNTF
Endothelial Cell Proliferation	NR	+	NR	NR	NR	NR
Tumor Inhibition	+	+	NR	+	NR	NR
Embryonic Stem Cell Maintenance	+	+	NR	NR	NR	NR
Hematopoietic Leukemic Cell Differentiation	+	+	+	+	+	NR
Melanoma Cell Inhibition	-	+	-	+	NR	NR
Neutrophil Proliferation/Stimulation	NR	NR	+	+	+	NR
Myoblast Proliferation	+	NR	NR	NR	NR	NR
Bone Remodeling	+	NR	NR	+	NR	NR
Kidney Development	+	NR	NR	NR	NR	NR
Neuronal Differentiation	+	+	NR	+	NR	+
Hepatocyte Stimulation	+	+	NR	+	NR	NR
Megakaryocyte Augmentation	+	+	-	+	+	NR
T-Cell Proliferation	NR	NR	NR	+	NR	NR
Keratinocyte Proliferation	NR	NR	NR	+	NR	NR
B-Cell Proliferation/Stimulation	NR	NR	NR	+	+	NR
Binding to Human Placental Cell Receptor	+	+	-	-	NR	NR
Hemopoietic Proliferation (Normal)	NR	NR	NR	+	+	NR
Neuron Augmentation	NR	NR	NR	NR	NR	+

"+" = activity; "-" = no activity; and "NR" = not reported.

As shown in the foregoing table, the six cytokines exhibit different activities. For example, OSM and IL-6 inhibit the growth of melanoma cells; LIF and G-CSF do not. However, LIF, IL-6 and IL-11 and G-CSF differ in that LIF, IL-6 and IL-11 are capable of stimulating proliferation and differentiation of megakaryocytes. OSM binds to human placental cell receptor; IL-6 does not; CNTF, IL-6, OSM and LIF stimulate neuronal differentiation, G-CSF may not.

Although some cytokines may enhance immune system health and white blood cell replacement in patients with depleted lymphocyte populations (*e.g.*, patients undergoing radiation or chemotherapy) or exhibit other desireable biological activity, each cytokine discussed above has a structure unique for intended function(s). Biological mechanisms are known to be highly selective and responsive only when intricate signaling systems invoke particular biochemical responses at a cellular (biochemical) level. Thus, each cytokine targets a specific cell receptor, inducing a particular biological reaction, producing a specific biological result.

These cytokines, CNTF, G-CSF, IL-6, IL-11, LIF and OSM, utilize specific mechanisms for effecting identified cellular responses. Their structural and biochemical specificity do not suggest that alternative cytokines, radically different from these cytokines, would have unexpected advantages in selective biological mechanisms over a much broader range of biological activities. One attempt to provide a therapeutic compound with multiple cytokine function resulted in a fusion protein, PIXY321, having an entire granulocyte macrophage colony stimulating factor (GM-CSF) cytokine, a linking sequence and an entire interleukin-3 (IL-3) cytokine. The extremely large polypeptide (spanning more than two cytokines) has both GM-CSF and IL-3 activity yet is difficult to produce and administer.

Summary of the Invention

The invention provides a group of therapeutic hybrid cytokines, having a size ranging from about 10 to about 30 kDa, which comprise portions of cytokines: leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF), interleukin-6 (IL-6), interleukin-11 (IL-11), oncostatin-M (OSM), and ciliary neurotrophic factor (CNTF).

IL-6, G-CSF, LIF, IL-11, CNTF and OSM each comprise four α-helical sequences. In each cytokine, the four α-helical sequences are linked by non-α-helical "linking" sequences of about 5-100 amino acids, and in some cases the α-helices are maintained in the proper conformation and geometry with respect to each other through disulfide bridges.

The inventive hybrid cytokines comprise three or four α-helical sequences selected from α-helical sequences of IL-6, G-CSF, LIF, IL-11, CNTF and OSM and linking sequences, ranging from about 5-40 amino acids in length, selected from the linking sequences of IL-6, G-CSF, LIF, IL-11, CNTF and OSM or other desirable linking sequences. In the hybrid cytokines, at least one α-helical sequence is derived from a different cytokine than at least one other α-helical sequence; or, at least one linking sequence of a cytokine differentiates the

inventive hybrid cytokine from a corresponding cytokine. In hybrid cytokines having three α-helical sequences selected from the group of cytokines listed, a possible fourth sequence may not correspond to any α-helical sequence in any of the cytokines. Thus, not all hybrid α-helical or linking sequences must derive from the same cytokine α-helical and linking sequences.

As referred to herein, cytokine refers to the amino acid or a DNA sequence encoding the cytokine amino acid sequence of human G-CSF, LIF, IL-6, OSM, CNTF or IL-11, which have been published. DNA sequences and corresponding amino acid sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, respectively. Each sequence listing illustrates α-helical and linking sequences for each corresponding cytokine.

Preferably, hybrid cytokine α-helical sequences are selected from corresponding α-helical sequences in the cytokine. "Corresponding" means that a first α-helical sequence of the hybrid cytokine corresponds to a first α-helical sequence from any cytokine, a second α-helical sequence of the hybrid cytokine corresponds to a second α-helical sequence from any cytokine, and a third α-helical sequence and possibly, but not necessarily, a fourth α-helical sequence of the hybrid cytokines correspond to third and fourth α-helical sequences, respectively, from any cytokine.

Inventive hybrid cytokines also contain linking sequences connecting α-helical sequences. The linking amino acid sequences preferably range from about 5 to 40 amino acids and are selected from the linking sequences of each cytokine. The linking amino acids are derived from linking sequences of the group of cytokines. Linking sequences are amino acid sequences (and DNA sequences encoding such amino acid sequences) which facilitate modification and assembly of nucleotide sequences used to express the hybrid cytokines and may, preferably, include amino acid sequences corresponding to nucleotide sequences having a resstriction site, for which restriction enzymes are available. A linking sequence preferably may be selected from a corresponding linking sequence in a cytokine. Again, "corresponding" means that a first hybrid linking sequence corresponds to a first linking sequence from a cytokine and a second hybrid linking sequence corresponds to a second linking sequence from any cytokine, and so on. Hybrid cytokines may include linking sequences not present in a cytokine, but which contain a restriction site to facilitate assembly of the DNA sequence encoding a hybrid cytokine.

The invention further provides: nucleotide sequences (and degenerate nucleotide sequences) encoding any inventive hybrid cytokine; expression systems capable of expressing the nucleotide sequences into hybrid cytokines; host cells transformed with these expression systems; and methods for recombinantly producing hybrid cytokines, in addition to, pharmaceutical compositions comprising the inventive hybrid cytokines and pharmaceutically acceptable excipients and/or carriers.

Brief Description of the Drawings

Figure 1 shows the organization of the four α-helical sequences deduced for the OSM, LIF, G-CSF and IL-6 cytokines. Also shown in this figure is an α-helical sequence organization for growth hormone which has been confirmed by X-ray crystallography.

Figure 2 illustrates biological activity data on 7TD1 cell proliferation for seven hybrid cytokines: LLLI; LLLIα; IIILα; IIIL; IIIG, IGGI, as compared against cytokine and mock assays.

Figure 3 shows a dose response curve of hybrid cytokine IGGI and cytokines IL-11 and IL-1 on 7TD1 cell proliferation, a measure of IL-6 biological activity.

Figure 4 illustrates biological activity assay of recombinant IL-6 and various inventive hybrid cytokines obtained from culture medium of Vero E6 cells stably transfected with varioius cytokine expression plasmids. Non-transfected culture medium served as a control.

Detailed Description of Preferred Embodiments

Names of hybrid cytokines are governed by the following conventions: Each hybrid cytokine name may have no less than three characters (a hybrid cytokine name may have more than three characters, depending on the number of α-helical sequences and the particular linking sequences present in the hybrid). In each name, G represents G-CSF; L represents LIF; I represents IL-6; O represents OSM, E represents IL-11 and C represents CNTF. Upper case letters designate α-helical sequences. Lower case letters (whether Arabic numerals, alphabetic or symbolic) immediately following a series of uppercase characters indicate a specific linking sequence. Hybrid cytokine names may include an integer (from 1 to 4) following each uppercase character representing a first, second, third or fourth cytokine α-helical sequence. This trailing integer indicates the location of the α-helical sequence in the originating cytokine. If the location of the α-helical sequence in the hybrid cytokine originates from the same position in a cytokine (*i.e.*, first, second, third or fourth), the character identifying the respective sequence will not have an integer following it.

Thus, for example, a hybrid cytokine having hybrid α-helical sequences I and IV corresponding to α-helical sequences I and IV of G-CSF, hybrid α-helical sequences II and III corresponding to α-helical sequences II and III of IL-11 and hybrid linking sequences I/II, II/III and III/IV, corresponding to linking sequences in G-CSF, IL-11 and G-CSF, respectively, would have a representative name: GEEGε. No integers follow any sequence character because each α-helical sequence corresponds to an α-helical sequence in a cytokine. The ε symbolic following uppercase letters specifying a hybrid α-helical sequence indicates a unique linking sequence in the hybrid cytokine. Because different linking sequences may occur in hybrid cytokines having identical α-helical sequences, lower-case characters following uppercase characters (and possibly integers) uniquely identify a particular linking sequence. A hybrid cytokine represented by the name C4C3EXβ would have the following sequence origins. Hybrid α-helical sequences I and II originate from the fourth and third α-helical sequences of

CNTF, respectively. The third hybrid α-helical sequence corresponds to the third α-helical sequence of IL-11. The fourth hybrid α-helical sequence is represented with an "X," indicating that this α-helical sequence does not originate from any cytokine, G-CSF, LIF, IL-6, IL-11, OSM or CNTF. The symbol β indicates a specific linking sequence for this hybrid cytokine. For example, β may represent that linking sequences I/II and II/III originate from the fourth and third linking sequences of CNTF, respectively, and that a third hybrid linking sequence (III/IV) does not have an origin from any cytokine.

Inventive hybrid cytokines comprise portions of cytokines: leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF), interleukin-6 (IL-6), interleukin-11 (IL-11), oncostatin-M (OSM), and ciliary neurotrophic factor (CNTF).

Preferably, the inventive hybrid cytokines comprise three to four α-helical sequences selected from α-helical sequences of IL-6, G-CSF, LIF, IL-11, CNTF and OSM. In the hybrid cytokine, at least one α-helical sequence is derived from a different cytokine than at least one other α-helical sequence. In hybrid cytokines having three α-helical sequences selected from the group of cytokines listed, a possible fourth sequence may not correspond to any α-helical sequence in any of the cytokines. Thus, not all α-helical sequences must derive from a cytokine α-helical sequence.

Preferably, hybrid cytokine α-helical sequences are selected from corresponding α-helical sequences in the cytokine. Each α-helical sequence may be interrupted by one or more non-helical sequences comprising from 8-30 amino acids.

Exemplary amino acid sequences for human cytokines are represented below in Sequence ID NOS: 1-6. The hybrid cytokines of the invention derive amino acid sequences from at least two of the related factors--leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF), interleukin-6 (IL-6), interleukin-11 (IL-11), ciliary neurotrophic factor (CNTF) and oncostatin-M (OSM). The amino acid sequences of each cytokine in human and some other species is known. The encoding genes have been cloned and reported in the literature. Human and murine genes encoding LIF are reported by Moreau *et al.*, Nature (1988) 336:690-692 and Simpson *et al.*, Eur. J. Biochem. (1988) 175:541-547. Nagata *et al.*, EMBO J. (1986) 5:575-581 and Tsuchiya *et al.*, Proc. Natl. Acad. Sci. USA (1986) 83:7633-7637, report human and murine G-CSF. Yasukawa *et al.*, EMBO J. (1987) 6:2939-2945 and Tanabe *et al.*, J. Immunol. (1988) 141:3875-3881 report human and murine IL-6. Malik *et al.*, Mol. Cell Biol. (1989) 9:2847-2853 disclose sequences for human OSM and Bruce *et al.*, Prog. Gr. Fac. Res. (1992) 4:157-170 discuss encoding sequence genes for simian OSM. Lam *et al.* Gene (1991) 102:271-276 report human CNTF; Stockli *et al.*, Nature (1989) 342:920-923 disclose rat CNTF; McKinley *et al.*, Genomics (1992) 13:814-819 discuss human IL-11; and Paul *et al.*, PNAS (1990) 87:7512-7516 report simian IL-11.

Alignments for the amino acid sequences of cytokines LIF, OSM, G-CSF, IL-6, IL-11 and CNTF, based on their predicted secondary structures, were conducted using a number of

software packages including PatMat software (Henikoff *et al.*, Methods Enzymol. (1990) 183:111-132); GenPro software (Riverside Scientific, Seattle, WA); P/C Gene software (Intelligenetics, Inc., Mountain View, CA); Scor Edit (J. Durand, Seattle, WA); Motif Program (Smith, Proc. Natl. Acad. Sci. USA (1990) 87:826-830), as implemented in the Protomat/Motif J software (Henikoff, Seattle, WA); and MacDNASIS software (Hitachi Software Engineering America, Ltd., San Bruno, CA).

Application and interpretation of these programs resulted in predicted secondary structures for the amino acid sequences of cytokines which comprise components of the inventive hybrid cytokines.

The results of this work are shown in Figure 1. As shown in Figure 1, each of LIF, G-CSF, IL-6, and OSM contain four α-helical sequences, numbered I-IV. The schematics also illustrate disulfide bridges; OSM and LIF having similar disulfide bridge locations. As shown, OSM and G-CSF have similar structures with LIF and IL-6, respectively. The disulfide bond linking a fourth α-helical sequence and a linking sequence between the first and second α-helical sequences determined in LIF and OSM is similarly found in the structure of growth hormone.

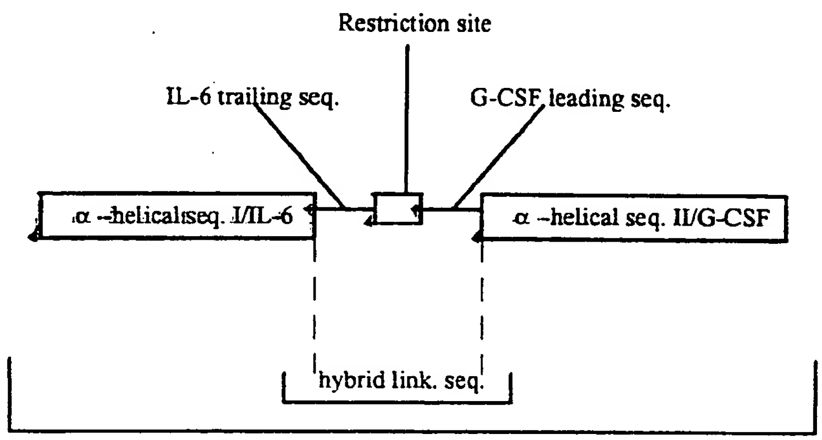
In human OSM, the α-helical sequence I extends approximately from amino acid residue 11-32; α-helical sequence II from residue 78-99; α-helical sequence III from residue 105-131; and α-helical sequence IV from residue 157-184. The locations of the various sequences of α-helices for human forms of cytokines are shown in Table II, wherein the start or end of an α-helical sequence listed below.

TABLE II

CYTOKINE	α-HELICAL SEQUENCE (amino acid residue #) I	II	III	IV
OSM	11-32	78-99	105-131	157-184
LIF	19-46	72-97	108-133	154-180
G-CSF	11-41	71-95	102-124	144-173
IL-6	24-48	80-104	111-133	157-183
IL-11	18-40	54-93	103-131	150-175
CNTF	16-44	62-98	102-134	151-181

As implied in the table, various α-helical sequences in each case will be linked by non-helical amino acid sequences, designated herein "linking sequences." Amino acid linking sequences preferably range from about 5 to 40 amino acids. Linking sequences are derived from linking sequences of cytokines or are spliced to insert a restriction site to facilitate

assembly of the hybrid cytokine. Linking sequences are designed to functionally and sterically space apart and properly orient α-helical regions of the resulting hybrid cytokines. A linking sequence may be selected from a corresponding linking sequence in a cytokine. Thus, a linking sequence I/II in the hybrid may be derived from linking sequence I/II of one of the G-CSF, OSM, LIF, IL-6, IL-11 and CNTF. Hybrid cytokines may include linking sequences not precisely present in a cytokine. Linking sequences themselves may be hybrids of linking sequences from two or more cytokines. For example, a portion of a linking sequence which trails a first α-helical sequence of IL-6 may combine with a portion of a linking sequence leading a second α-helical sequence of G-CSF, the trailing portion and leading linking sequences joined by a hybrid sequence which includes a restriction site to facilitate assembly of the hybrid cytokine. Graphically represented, a hybrid cytokine would appear, in relevant part, as:



HYBRID CYTOKINE

Linking sequence I/II in OSM, for example, extends from residue 33-77; linking sequence II/III from position 100-104; and linking sequence III/IV from position 132-156. Locations of various sequences of linking sequences for human forms of six cytokines are shown in Table III, wherein the start or end of a linking sequence may vary by as many as five amino acids from the consensus start and end of linking sequences listed below. Linking sequence I/II corresponds to an amino acid sequence between α-helical sequences I and II; linking sequence II/III, between α-helical sequences II and III; and linking sequence III/IV, between α-helical sequences III and IV.

TABLE III
LINKING SEQUENCES
(amino acid residue #)

CYTOKINE	I/II	II/III	III/IV
OSM	33-77	100-104	132-156
LIF	47-71	98-107	134-153
G-CSF	42-70	96-101	125-143
IL-6	49-79	105-110	134-156
IL-11	42-53	94-102	132-149
CNTF	45-61	99-101	135-150

A hybrid cytokine polypeptide comprises a first, second, third and preferably a fourth α-helical sequence. Each α-helical sequence is derived from an α-helical sequence of cytokines LIF, G-CSF, IL-6, IL-11, OSM or CNTF, preferably a corresponding α-helical sequence. Therefore in inventive hybrid cytokines, at least one of these three or four α-helical sequences is derived from a different cytokine.

Preferred inventive hybrid cytokines have α-helical sequences derived from cytokines of the species intended for use. For human therapy, all four α-helical sequences are derived from human forms of cytokines (preferably, the inventive hybrid cytokines comprise human α-helical and linking sequences). For veterinary use, for example bovine, all α-helical sequences are derived from bovine cytokine sequences.

Preferred hybrid cytokines have either α-helical sequences I, II, and III from the same cytokine and IV from a different cytokine, or conversely, sequences II, III and IV are from the same cytokine and sequence I is from a different one. In general, it is preferred that hybrid sequences II and III originate from the same cytokine.

Of particular significance: in all six cytokines, α-helical sequences I and IV have opposite N→C orientations. Similarly, α-helical sequences II and III have opposite polarity N →C orientations. In more preferred hybrid cytokines, relative polarity orientation of hybrid sequences I-IV maintains a natural pattern, in particular, hybrid sequences I and IV, with respect to each other.

In OSM and LIF, length of linking sequence I/II and linking sequence III/IV permit sequences I and IV to have the same N→C orientation as sequences II and III, respectively. Similarly, in G-CSF and IL-6, α-helical sequences I and II and III and IV to have parallel N→C orientations. However, a "double negative" for G-CSF and IL-6 results in the same relative sequence I and IV orientations in G-CSF and IL-6, as is the case in OSM and LIF.

Thus, preferred hybrid cytokines have both linking sequences I/II and III/IV longer (about 20-30 amino acids), permitting parallel orientation of joined helical sequences as in LIF

and OSM. Linking sequences may be selected so as to assure anti-parallel orientation of α-helical sequences I and IV in all cases.

Particularly preferred hybrid cytokines comprise: 1) first and fourth α-helical sequences derived from the same cytokine, 2) second and third α-helical sequences derived from the same cytokine; 3) at least one sequence derived from IL-6; or 4) with respect to α-helical sequence origins, have α-helical sequences I-IV as represented below.

GGGI; OOOI; LLLI; IIIO; GGGO; OOOG; LLLO; IIIG; GGGL; OOOL; LLLG; IIIL; IGGG; IOOO; ILLL; OIII; OGGG; GOOO; OLLL; GIII; LGGG; LOOO; GLLL; LIII; GLLG; GIIG; IGGI; LOGI; LLII; LLGG; IIGG; EGGG; OOOE; LLLE; IIIE; LEEE; CEEE; ECCC; EEEC; GCCC; CCCE; LLLC; OOOC; IIIC; GCCG; CGGC; LCCC; CCLL; CCII; CGGG; CELI; and ECCE.

And, especially preferred hybrid cytokines include, but are not limited to:

GGLL; GGII; GGOO; GGGI; IGGG; GILO; LOGI; LLII; GGGO; GGGL; OOOG; LLLG; GOOO; OGGG; LGGG; GGGI.

In each preferred hybrid cytokines, each hybrid α-helical sequence has a corresponding sequence in a cytokine, therefore, no integers show position origin. The α-helical sequences shown are linked through non-helical linking sequences of 5-40 amino acids. Preferred hybrid cytokines have linking sequences derived, even if only partially, from corresponding linking sequences of cytokines. However, other preferred hybrid cytokines may or may not have linking sequences derived from corresponding cytokines. These preferred hybrid cytokines may alternatively have linking sequences selected to maintain a hybrid three-dimensional, α-helical sequence conformation similar to the originating cytokines. In the hybrid cytokines previously shown, linking sequences may preferably be derived from corresponding linking sequences of a cytokine. Thus, for example, a linking sequence between α-helical sequence I and II is derived from the linking sequence I/II of the same cytokine:

Because disulfide linkages, as shown in Figure 1, are thought to contribute to maintaining conformation, preferred suitable linking of α-helical sequences provide at least two cysteine residues, furnishing appropriate disulfide links.

<u>Hybrid Cytokine Synthesis</u>

Inventive, hybrid cytokines may be made using known solid-phase peptide synthesis techniques combined with linking technology. However, synthesis of appropriate-length peptides is laborious and may be difficult. Subsequent to peptide synthesis, proper conditions are required to effect three-dimensional folding of synthesized peptides to assume α-helical and

tertiary conformations discussed above. Alternatively, inventive hybrid cytokines may be prepared using recombinant DNA techniques.

Recombinant DNA technology for producing desired proteins is known by ordinarily skilled artisans, including provision of a coding sequence for a desired protein, the coding sequence operably linked to DNA sequences capable of effecting its expression. It may be desirable to produce the hybrid cytokines as fusion proteins, freed from upstream, downstream or intermediate sequences, or as proteins linked to leader sequences, effecting secretion of a desired cytokines into cell culture medium.

A DNA-based expression system will also contain "control sequences" necessary for transcription and translation of a message. Known, required expression components, include constitutive or inducible promoter systems, translational initiation signals (in eucaryotic expression), polyadenylation translation termination sites, and transcription terminating sequences. Host vectors containing controls which permit operable linking of desired coding sequences to required control systems are known by artisans ordinarily skilled in recombinant technology. Such vectors can be found which are operable in a variety of hosts. High stringency, as used herein, means the integrity of the originating double-helix DNA structure is maintained in the recombinant DNA.

Inventive hybrid cytokines may be produced in procaryotic cells using appropriate controls, such as *trp* or *lac* promoters, or in eucaryotic host cells, capable of effecting post-translational processing that permits proteins to assume desired three-dimensional conformation. Eucaryotic control systems and host vectors are known; including the *leu* and glycolytic promoters useful in yeast, the viral SV40 and adenovirus and CMV promoters in mammalian cells, inducible promoters such as the metallothionein promoter also suitable for mammalian cells, and the baculovirus system which is operable in insect cells. Plant vectors with suitable promoters, such as the *nos* promoter have also been used successfully.

Hybrid cytokines may be prepared in procaryotic as well as eucaryotic hosts. Although generally glycosylated in their native forms, glycosylation is known not to be essential for an activity of any of the foregoing six cytokines. Suitable refolding conditions, as understood by ordinarily skilled artisans, may provide alternative techniques for obtaining a desired conformation.

Standard laboratory manuals (*e.g.*, Sambrook *et al.* published by Cold Spring Harbor Laboratories, Cold Spring Harbor, NY), present standard techniques and methodologies for expressing DNAs encoding a desired protein, culturing appropriate cells, providing suitable expression conditions, and recovering a resulting protein from culture. In preparing the inventive hybrid cytokines, a suitable DNA encoding the desired hybrid, constructed utilizing any of the foregoing techniques, is operably linked to control sequences in a suitable expression system which is then transformed or transfected into a compatible host. Host cells are cultured using conditions appropriate for growth, expression of the desired hybrid cytokine being preferably induced after some predetermined growth level has occurred. Hybrid cytokine

production is monitored and the desired hybrid collected from culture either from a supernatant or by first lysing host cells with an appropriate agent.

Procedures analogous to those employed for purifying cytokines may be used to purify the isolated hybrid cytokine for use in therapeutic or diagnostic compositions.

Preparation of Antibodies

Antibodies specifically reactive with the hybrid cytokines of the invention or immunoreactive fragments of these antibodies may be prepared using standard immunization protocols. These may be utilized as polyclonal antisera or the spleen cells or peripheral blood lymphocytes of the immunized animals may be immortalized to obtain isolated cell cultures which produce monoclonal antibodies specific for these hybrids. The antibodies may be used intact, or as fragments such as Fab, Fab' or F(ab')2 fragments. As the hybrid cytokines are relatively large proteins, it should not be necessary to enhance their immunogenicity by conjugation to carrier; however, such enhancement is possible and construction of such conjugates is well known in the art.

Thus, the hybrid cytokine, optionally conjugated to an immunological carrier, is administered in a standard immunization protocol with or without the use of adjuvant to a suitable subject, usually rats, sheep, or rabbits. Antibody formation is monitored by titrating the serum using the cytokine as antigen and employing standard immunoassay techniques. When high titers are achieved, the sera can be used per se or the spleen cells or peripheral blood lymphocytes isolated and immortalized, for example, using the fusion technique of Kohler and Millstein to provide immortalized cells capable of secreting the desired monoclonal antibodies. Individual clones of these immortalized cells are then screened, again using standard immunological techniques, for those colonies which secrete antibodies specifically immunoreactive with the hybrid cytokine immunogen.

The antibodies prepared in the foregoing manner or fragments thereof are useful in diagnostic assays for monitoring the pharmacokinetics and progress of therapeutic regimens using the hybrid cytokines of the invention. Thus, the dosage levels of the hybrid cytokines in the therapeutic applications set forth below can be regulated according to the metabolic fate of the previously administered dosages.

Administration and Utility

The invention further provides: nucleotide sequences encoding any inventive hybrid cytokine; expression systems capable of expressing the corresponding nucleotide sequences; host cells transformed with these expression systems; and methods for recombinantly producing hybrid cytokines. The invention permits pharmaceutical compositions comprising the inventive hybrid cytokines, polypeptides and/or pharmaceutically acceptable excipients and/or carriers. The invention permits antibodies or fragments specifically immunoreactive with these hybrid cytokines.

The hybrid cytokines of the invention are useful in treating the indications for which their native counterparts are often employed. However, the hybrid forms of the cytokines possess unique properties which make them suitable alternatives in the methods and procedures commonly employed with respect to the native molecules. For example, a hybrid cytokine composed of α-helical sequences from two different cytokines may have biological activity of both cytokines.

In addition, some of the hybrid cytokines are capable of binding the receptors ordinarily bound by the native molecules but fail to activate these receptors. These forms of the hybrid cytokines are, thus, antagonists. These may be useful in treating conditions where presence of the cytokne that ordinarily binds to the receptor is responsible for undesired cell proliferation. For example, IL-6 and OSM are known to be present in high levels with Kaposi's sarcoma. These are found also in high concentrations in the synovial fluid from patients suffering from rheumatoid arthritis. In these conditions, the hybrid cytokine antagonists are particularly useful.

Conversely, the hybrid cytokines which are agonists can be employed in circumstances wherein the cytokines are often used. For instance, these agonist hybrid cytokines may be used in liver cell regeneration and in *in vitro* fertilization procedures to enhance these processes.

The hybrid cytokine may possess properties exhibited by neither of its components taken alone. It is known, for example, that co-administration of LIF and G-CSF results in a synergistic effect in inhibiting colony formation and inducing differentiation of human U937 and HL60 myelocytic leukemia cell lines although neither alone has this effect (Maekawa *et al.*, Leukemia (1989) 3:270-276.) Similarly, we have found that although neither LIF nor OSM inhibit colony formation of U937, when supplied in combination, at 10 ng/ml using 300 cells in soft agar, more than 60% inhibition of colony formation is obtained.

Thus, combination of the α-helical sequences from more than one growth factor results in hybrid cytokines with a unique spectrum of properties. These inventive hybrid cytokines are useful generally in inhibiting tumor proliferation, in bone remodeling, in stimulating the growth of desired cells, such as neurites or T-cells, and in enhancing the differentiation of hematopoietic cells. Hybrid cytokines are therefore highly useful in direct treatment of malignancies. The inventive hybrid cytokines are especially useful in maintaining the general health and immune capacity of a subject undergoing cytoreductive therapy (radiation therapy or chemotherapy) for such indications.

The selection of particular conditions or procedures suitable for the hybrid cytokine in question depends, of course, on its particular agonist or antagonist activity spectrum.

The properties of a particular hybrid can be ascertained through standard *in vitro* tests known in the art. Such tests include those, for example, which show: 1) induction of differentiation into macrophages (Tomita *et al.*, J. Biol. Chem. (1984) 259:10978-10982); 2) enhancement of interleukin-3-dependent colony formation of primitive blast cells (Leary *et al.*, Blood (1990) 75:1960-1964); 3) promotion of megakaryocyte growth and differentiation (Metcalf *et al.*, Blood (1990) 76:50-56); 4) induction of neuronal differentiation (Yamamuri *et*

al., Science (1989) 246:1412-1416); and 5) induction of bone resorption (Ishimi *et al.*, J. Immunol. (1990) 145:3297-3303). A large number of *in vitro* assays indicating an ability of cytokines to stimulate growth and differentiation of desired cells and inhibit the growth of undesired malignant cells is known in art. Animal model systems can also be used to verify unique, unexpected spectrum of properties associated with each hybrid cytokine.

Particularly useful *in vitro* tests which can be used to confirm the spectrum of activity of the hybrid cytokines include, but are not limited to the: 1) inhibition of DNA synthesis in M-1 myeloid leukemic cells; effect on growth of human A-375 melanoma cells (Zarling *et al.*, Proc. Natl. Acad. Sci. USA (1986) 83:9739-9743); or effect of these cytokines on embryonic stem cells cultured *in vitro* (Smith *et al.*, Devel. Biol. (1987) 121:1-9 and Williams *et al.*, Nature (1988) 336:684-687).

The foregoing procedures can be adapted to assess both agonist and antagonist behavior. In assessing antagonist behavior, a hybrid cytokine may be used in the presence of a known agonist and its effect on the activity of the known agonist is assessed.

As set forth above, the hybrid cytokines of the invention are applicable to *in vivo* and *in vitro* procedures involving both human and animal cells. They are suitable for both medical and veterinary use.

For therapeutic use, the hybrid cytokines of the invention are formulated into standard pharmaceutical compositions suitable for the administration of proteins. Suitable formulations can be found, for example, in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Company, Easton, PA. Comparable compositions for veterinary use are also known in the art. Generally, administration is systemic, usually by injection, such as intravenous or intramuscular injection or can be effected by transdermal or preferably transmucosal delivery. Suitable formulations for effecting transmucosal delivery include, for example, various detergents and bile salts or fusidic acid derivatives. The dosage levels of the hybrid cytokines of the invention are comparable to those useful for the native molecules. These levels are understood in the art, and the precise dosage can be adjusted according to the condition of the patient, the mode of administration, and the judgment of the attending physician.

The hybrid cytokines of the invention may also be labeled using suitable fluorometric, colorimetric, enzymic, or radioactive labels for use in assays to ascertain the formation of antibodies in patients being treated. Nucleotide base and amino acid sequence listings for cytokines and inventive hybrid cytokines appear below. The invention, illustrated by the following examples, should not be deemed as limited in any way by these representative examples.

Example 1

cDNA clones for the human cytokines G-CSF and IL-6 were obtained by PCR amplification (Kawasaki, PCR Protocols, A Guide to Methods and Applications, pp. 21-27 Academic Press, 1990) using primers based upon published sequences. The sequence of the

cDNA encoding the mature protein of each cytokine was joined to synthetic DNA fragments encoding the signal peptide sequence derived from *E. coli* alkaline phosphatase and the FLAG® octa-peptide sequence (Hopp *et al.*, BioTechnology (1988) 6:1204-1210). The resulting fragment, with a unique *Kpn* I site and *BamH* I site at its 5' end and 3' end, respectively, was inserted between the *Kpn* I and *BamH* I sites, within the multiple cloning site of the mammalian expression vector pBL3. pBL3 was derived from mammalian episomal expression vectors pMEP4 and pCEP4 (Invitrogen Corp.). Specifically, a 600 bp *Spe* I -- *Kpn* I fragment spanning the CMV promoter from pCEP4 and a 9500 bp *Xba* I -- *Kpn* I vector fragment from pMEP4 were isolated and ligated together to form pBL3.

Using the IL-6 and G-CSF cDNA sequences prepared and obtained as described above, a hybrid cytokine, IGGGγ (SEQ ID NO:7, below), was prepared having sequences from both cytokines. An alkaline phosphatase signal sequence for protein secretion extends from amino acid residues -30 to -10. The FLAG® octa-peptide sequence for recombinant protein detection and cleavage extends from amino acids -9 to -3. A *Hind* III restriction site was positioned between the FLAG® sequence and the start of the hybrid cytokine mature protein sequence. The first 42 amino acids of this hybrid cytokine were derived from human IL-6 and amino acids 43-181 were derived from human G-CSF. The IGGGγ construct was made by fusing the IL-6 and G-CSF cDNA at a precise location in the hinge sequence between helix 1 and helix 2 domains using PCR (PCR Protocols, *supra* at pp. 177-183).

Plasmid pBL3 was used to express the hybrid cytokine. pBL3 was transfected into 293-EBNA cells (available from Invitrogen Corp.) using cationin lipid DOTAP® according to the manufacturer's procedure (Boerhinger Mannheim). The cell culture media were changed one day after transfection to remove excess DOTAP®. The cell culture media were then collected two days later to obtain the inventive IGGGγ hybrid cytokine for a biological activity assay.

Biological acivity of the hybrid cytokine was based on an assay useful in determining an ability of test samples to support growth of 32D cells, a G-CSF-dependent cell line. Test samples of isolated hybrid cytokines were added to cell culture media at different concentrations and incubated for three days. Biological activity was determined by measuring viability (in %) of the 32D cells using a trypan blue exclusion staining technique. A comparison of the activity of the hybrid cytokine IGGGγ to human G-CSF suggests a % Viability about 5 to 10% of values obtained for G-CSF activity. Table IV reports % Viability assay results for various test samples:

TABLE IV

Test Samples	% Viability
RPMI media (neg. control)	4.0
G-CSF 10 ng/ml	21.8
G-CSF 25 ng/ml	34.9
G-CSF 50 ng/ml	42.0
G-CSF 100 ng/ml	41.1
Mock transfected DNA control 1.0%	6.1
Mock transfected DNA control 2.5%	4.0
Mock transfected DNA control 5.0%	3.0
Mock transfected DNA control 10.0%	5.0
IGGGγ 1.0%	2.0
IGGGγ 2.5%	8.9
IGGGγ 5.0%	7.7
IGGGγ 10.0%	6.2

EXAMPLE 2

Hybrid Cytokines LLLI, LLLIα, IIILα, IIIL, IIIG, IGGI (SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19 SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23, respectively) were made via transient transfection into 293-EBNA cells (mammalian expression system) according to a procedure akin to the procedure described in Example 1.

(IL6.1, LIF.1) and (IL11.3) are plasmids expressing type IL-6, LIF and IL-11 cytokines, respectively. LLLI and LLLIα refer to plasmids expressing hybrid cytokines LLLI and LLLIα, respectively. The first three α-helical sequences of LLLI and LLLIα were derived from LIF and the fourth α-helical sequence was derived from IL-6. LLLIα has two additional amino acids inserted in a linking sequence between α-helices III and IV, as compared with LLLI, which has no additional amino acids at this location. IIILα and IIIL are plasmids expressing hybrid cytokines, having the first three α-helical domains derived from IL-6 and the fourth α-helical domain derived from LIF. IIILα has an additional 2 amino acids inserted in the hinge sequence between α-helices III and IV when compared to IIIL. IIIG is a plasmid expressing a hybrid cytokine having the first three α-helical sequences derived from IL-6 and the fourth α-helical sequence from G-CSF. IGGI is to a plasmid expressing a hybrid cytokine with the first and fourth α-helical sequences derived from IL6 and the second and third α-helical sequences derived from G-CSF.

Each transfection was assayed in a 7TD1 assay, an assay based on the capability of the test samples to support the growth of an IL6-dependent cell line, 7TD1, obtained from ATCC (American Type Culture collection), according to the following procedure.

To quantify the significance of the 7TD1 assay results, Western blot analyses of the various tested samples were conducted to determine the degree of expression of the hybrid cytokines as compared with cytokines. An anti-FLAG® antibody was used to measure protein expression of each recombinant polypeptide. Results obtained establish that hybrid cytokine expression levels were much lower than expression levels for IL-6, IL-11 and LIF cytokines. The data presented in Fig. 2, when interpreted in view of lower hybrid cytokine concentrations per unit of culture media tested, support a conclusion that the hybrid cytokines have activities equal to IL-6 or IL-11. The data presented in Fig. 2 should be interpreted accordingly.

Hybrid cytokine test samples were added to a culture media containing 7TD1 cells. The amount of viable 7TD1 cells was determined 72 hours later by staining the cells with a metabolic dye MTS® according to the manufacturer's (Promega) procedure. 7TD1 cells were resuspended in RPMI culture media + 10% FBS (fetal bovine serum) and plated in 96-titer plates. An indicator dye (MTS®) was added to the cells 2 to 3 days later. The cells were returned to a CO_2 incubator for 1 hour before determining an A490 using the plate reader, which is a colorimetric assay that uses the tetrazolium salt of MTS to report cell proliferation, viability and cytotoxicity. The indicator dye shows cell activity by serving as substrate for mitochrondrial dehydrogenases for the formation of soluble formazan dyes which are quantitated using a plate reader.

All hybrid cytokine and cytokine test samples stimulated 7TD1 proliferation, as compared with mock transfected control samples derived from conditioned media of 293-EBNA cells transfected without plasmid DNA. Samples with the highest activity were the ones derived from wild type IL-6 and IL-11. Figure 2 illustrates activity assay data for seven hybrid cytokines prepared and obtained in this example. Activities of inventive hybrid cytokines are about 40-50% of IL-6, IL-11 and LIF cytokines. In view of a lower degree of hybrid expression in this system, the activities of the hybrids most likely match activities of corresponding cytokines.

EXAMPLE 3

In view of the relatively poor expression of hybrid cytokines in a mammalian cell line, *E. coli* (prokaryotic cells) was used to express hybrid cytokine IGGI. Hybrid cDNA sequences encoding hybrid cytokine amino acid sequences were cloned according to the procedures used in Examples 1 and 2, above. The cDNAs flanked by a *Hind*III site and a *BamH*I site between the *Hind*III and *BamH*I site of an expression vector pT.His in such way that the cytokine cDNA was expressed under the control of a *tac* promotor as a fusion protein with methionine followed by 6 histidine residues at the N-terminus. *E. coli*, harboring expression plasmids for IL-11, IGGI and IL-1α, were induced with IPTG. The recombinant hybrid cytokine and cytokine polypeptides were purified from the cell extract using a His.Bind resin (Novagen) according to the manufacturer's procedure.

Figure 3 shows a dose response curve for *E. coli* produced hybrid cytokine IGGI, IL-11, and IL-1α on 7TD1 proliferation. SDS gel analysis indicates the 3 cytokines tested were expressed at similar levels in *E. coli*. Hybrid cytokine IGGI and cytokine IL-11 have higher activity when compared to IL-1α.

EXAMPLE 4

Using a method as described in the foregoing examples, inventive hybrid cytokines were prepared from cytokines IL-11, G-CSF, LIF and IL-6. Complete amino acid and nucleotide sequences for the cytokines appear below (SEQ ID NO:6, SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3).

Hybrid cytokine LLLE (SEQ ID NO:24), comprises sequences from LIF and IL-11 cytokines. Specifically, the hybrid sequence from 1 to 148 corresponds to a sequence derived from the first three α-helical sequences of LIF, and the hybrid sequence from 149 to 191 corresponds to sequence derived from the fourth α-helical sequence of IL-11. The hybrid cytokine IIIE (SEQ ID NO:25) comprises sequences from IL-6 and IL-11, specifically, the hybrid sequence from 1 to 139 corresponds to a sequence derived from the first three α-helical sequence of of IL-6, and the hybrid sequence from 140 to 183 corresponds to a sequence derived from the fourth α-helical sequence of IL-11. In yet another exemplary hybrid cytokine, GGGE (SEQ ID NO:26), the hybrid sequence from 1 to 133 corresponds to a sequence derived from the first three α-helical sequences of G-CSF, and the hybrid sequence from 134 to 177 corresponds to sequence derived from the fourth α-helical sequence of IL-11.

EXAMPLE 5

In this Example, hybrid cytokines, expressed in Examples 4-6 above, were used in assays designed to determine ability of inventive hybrid cytokines to stimulate the formation of cell colonies from mouse bone marrow cell cultures, as compared with cytokines.

In the assay, bone marrow cells, isolated from mouse femur, were cultured in the presence of various conditioned media (with appropriate cells for each respective assay) from Vero E6 cells transfected with expression vectors of various hybrid cytokines. In results shown in Table V, two hybrid cytokines, IIIG and IGGI, increased colony formation units (CFU--defined as the number of cell colonies formed) as compared with a control medium containing untransfected Vero E6 cells. IIIG transfected cells had CFU values nearly equivalent to values obtained for G-CSF transfected cells, prepared and tested in a like manner. This is surprising in view of the fact that only one of four α-helical sequences was derived from G-CSF.

TABLE V

SAMPLES	CFU.1	CFU.2	CFU.3	CFU.4	Avg.	St. Dev.
Control Medium	55	59	54	55	55.8	2.2
IIIG	87	77	93	n.a	85.7	8.1
IGGI	67	59	63	59	62	3.8
G-CSF	87	87	79	90	88	3.6

EXAMPLE 6

As in Example 2 above, hybrid cytokines IIILα, LLLIα, GGGE, LLLE, IIIE, IIIL, LLLI, IGGI and IIIG were assayed for proliferation of 7TD1 cells obtained from ATCC.

Figure 4 represents activity assay data of recombinant IL-6 and these various hybrid cytokines made from stablly transfected Vero E6 cells using culture medium from non-transfected cells as a control (no DNA). Culture media from IL-6 and two hybrid cytokines, IIIG and IGGI, transfected cells exhibited mitogenic proliferation activity for 7TD1 cells.

EXAMPLE 7

In a procedure similar to Example 5, hybrid cytokines IIIG and GGGE were prepared and assayed to determine their respective abilities to stimulate formation for CFU-GM from murine bone marrow. Human G-CSF has sufficient sequence homology with a corresponding murine G-CSF cytokine to obtain relevant data.

Fresh murine bone marrow cells were harvested from Balb/c female mouse femurs and cultured in media prepared for CFU-GM, including 1 percent of a spleen-conditioned medium. Hybrid cytokines, expressed as unpurified supernatants from transfected Vero E6 cells, were tested along with 100 ng/ml of rhG-CSF (from Collaborative Research) and the products of several transfections of unmodified human G-CSF cDNA.

Results shown in Figure 5 indicate that although GGGE had minimal activity and supernatants from two G-CSF transfections stimulated an increase of approximately 25-30 colonies above control (no DNA), IIIG showed as much activity as either of the two G-CSF transfections.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
  (i) APPLICANT:
    (A) NAME:Cell Therapeutics, Inc.
    (B) STREET:201 Elliott Avenue West, Suite 400
    (C) CITY:Seattle
    (D) STATE OR PROVINCE:Washington
    (E) COUNTRY:USA
    (F) POSTAL CODE:98119
  (i) APPLICANT:
    (A) NAME:Todaro, George J.
    (B) STREET:1940 Fifteenth Avenue East
    (C) CITY:Seattle
    (D) STATE OR PROVINCE:Washington
    (E) COUNTRY:USA
    (F) POSTAL CODE:98112
  (i) APPLICANT:
    (A) NAME:Leung, David W.
    (B) STREET:7625 East Mercer Way
    (C) CITY:Mercer Island
    (D) STATE OR PROVINCE:Washington
    (E) COUNTRY:USA
    (F) POSTAL CODE:98040
  (i) APPLICANT:
    (A) NAME:Rose, Timothy M.
    (B) STREET:5045 NE 70th Street
    (C) CITY:Seattle
    (D) STATE OR PROVINCE:Washington
    (E) COUNTRY:USA
    (F) POSTAL CODE:98115
  (ii) TITLE OF INVENTION:HYBRID CYTOKINES
  (iii) NUMBER OF SEQUENCES:26
  (iv) COMPUTER-READABLE FORM:
      (A) MEDIUM TYPE:3.5" diskette, 720K, DOS-formatted
      (B) COMPUTER:AST-IBM Compatible
      (C) OPERATING SYSTEM:MS-DOS Version 6
      (D) SOFTWARE:WORD for WINDOWS
  (v) CURRENT APPLICATION DATA:
    (A) APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:
  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH:525
    (B) TYPE:nucleic acid
    (C) STRANDEDNESS:double stranded
    (D) TOPOLOGY:linear
  (xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:

```
ACC CCC CTG GGC CCT GCC AGC TCC CTG CCC CAG AGC TTC CTG CTC AAG   48
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
                  5                  10                  15
```

```
CTG TGT GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG   96
Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
                20                  25                  30

GAG AAG TGC TTA GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG  144
Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
            35                  40                  45

CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC  192
Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
        50                  55                  60

CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC  240
Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
 65                  70                  75                  80

GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC  288
Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
                85                  90                  95

CCC GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC  336
Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
                100                 105                 110

TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT  384
Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
            115                 120                 125

GCC CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC  432
Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
        130                 135                 140

CAG CGC CGG GCA GGA GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC  480
Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
145                 150                 155                 160

CTG GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT GCC CAG CCC TGA      525
Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro ***
                165                 170                 175
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(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:540
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

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[illegible]
Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg His Pro
                5                   10                  15

TGT CAC AAC AAC CTC ATG AAC CAG ATC AGG AGC CAA CTG GCA CAG CTC   96
Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala Gln Leu
                20                  25                  30

AAT GGC AGT GCC AAT GCC CTC TTT ATT CTC TAT TAC ACA GCC CAG GGG  144
Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala Gln Gly
            35                  40                  45

GAG CCG TTC CCC AAC AAC CTG GAC AAG CTA TGT GGC CCC AAC GTG ACG  192
Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn Val Thr
        50                  55                  60
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GAC TTC CCG CCC TTC CAC GCC AAC GGC ACG GAG AAG GCC AAG CTG GTG 240
Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys Leu Val
 65                 70                  75                  80

GAG CTG TAC CGC ATA GTC GTG TAC CTT GGC ACC TCC CTG GGC AAC ATC 288
Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly Asn Ile
                 85                  90                  95

ACC CGG GAC CAG AAG ATC CTC AAC CCC AGT GCC CTC AGC CTC CAC AGC 336
Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu His Ser
            100                 105                 110

AAG CTC AAC GCC ACC GCC GAC ATC CTG CGA GGC CTC CTT AGC AAC GTG 384
Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser Asn Val
        115                 120                 125

CTG TGC CGC CTG TGC AGC AAG TAC CAC GTG GGC CAT GTG GAC GTG ACC 432
Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp Val Thr
    130                 135                 140

TAC GGC CCT GAC ACC TCG GGT AAG GAT GTC TTC CAG AAG AAG AAG CTG 480
Tyr Gly Pro Asp Thr Ser Gly Lys Asp Val Phe Gln Lys Lys Lys Leu
145                 150                 155                 160

GGC TGT CAA CTC CTG GGG AAG TAT AAG CAG ATC ATC GCC GTG TTG GCC 528
Gly Cys Gln Leu Leu Gly Lys Tyr Lys Gln Ile Ile Ala Val Leu Ala
                165                 170                 175

CAG GCC TTC TAG                                                 540
Gln Ala Phe ***
            180
```

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:540
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

```
GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC AGA CAG CCA CTC ACC TCT  48
Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr Ser
                  5                  10                  15

TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC ATC CTC GAC GGC ATC TCA  96
Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile Ser
             20                  25                  30

GCC CTG AGA AAG GAG ACA TGT AAC AAG AGC AAC ATG TGT GAA AGC AGC 144
Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn Met Cys Glu Ser Ser
         35                  40                  45

AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC CTT CCA AAG ATG GCT GAA 192
Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu
     50                  55                  60

AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT GAG GAG ACT TGC CTG GTG 240
Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val
 65                  70                  75                  80

AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG GTA TAC CTA GAG TAC CTC 288
Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu
                 85                  90                  95
```

CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA GCC AGA GCT GTC CAG ATG 336
Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met
100 105 110

AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG AAA AAG GCA AAG AAT CTA 384
Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu
115 120 125

GAT GCA ATA ACC ACC CCT GAC CCA ACC ACA AAT GCC AGC CTG CTG ACG 432
Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr
130 135 140

AAG CTG CAG GCA CAG AAC CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC 480
Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu
145 150 155 160

ATT CTG CGC AGC TTT AAG GAG TTC CTG CAG TCC AGC CTG AGG GCT CTT 528
Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu
165 170 175

CGG CAA ATG TAG 540
Arg Gln Met ***
180

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:591
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

GCG GCT ATA GGC AGC TGC TCG AAA GAG TAC CGC GTG CTC CTT GGC CAG 48
Ala Ala Ile Gly Ser Cys Ser Lys Glu Tyr Arg Val Leu Leu Gly Gln
5 10 15

CTC CAG AAG CAG ACA GAT CTC ATG CAG GAC ACC AGC AGA CTC CTG GAC 96
Leu Gln Lys Gln Thr Asp Leu Met Gln Asp Thr Ser Arg Leu Leu Asp
20 25 30

CCC TAT ATA CGT ATC CAA GGC CTG GAT GTT CCT AAA CTG AGA GAG CAC 144
Pro Tyr Ile Arg Ile Gln Gly Leu Asp Val Pro Lys Leu Arg Glu His
35 40 45

TGC AGG GAG CGC CCC GGG GCC TTC CCC AGT GAG GAG ACC CTG AGG GGG 192
Cys Arg Glu Arg Pro Gly Ala Phe Pro Ser Glu Glu Thr Leu Arg Gly
50 55 60

[illegible]
Leu Gly Arg Arg Gly Phe Leu Gln Thr Leu Asn Ala Thr Leu Gly Cys
65 70 75 80

GTC CTG CAC AGA CTG GCC GAC TTA GAG CAG CGC CTC CCC AAG GCC CAG 288
Val Leu His Arg Leu Ala Asp Leu Glu Gln Arg Leu Pro Lys Ala Gln
85 90 95

GAT TTG GAG AGG TCT GGG CTG AAC ATC GAG GAC TTG GAG AAG CTG CAG 336
Asp Leu Glu Arg Ser Gly Leu Asn Ile Glu Asp Leu Glu Lys Leu Gln
100 105 110

ATG GCG AGG CCG AAC ATC CTC GGG CTC AGG AAC AAC ATC TAC TGC ATG 384
Met Ala Arg Pro Asn Ile Leu Gly Leu Arg Asn Asn Ile Tyr Cys Met
115 120 125

```
GCC CAG CTG CTG GAC AAC TCA GAC ACG GCT GAG CCC ACG AAG GCT GGC 432
Ala Gln Leu Leu Asp Asn Ser Asp Thr Ala Glu Pro Thr Lys Ala Gly
    130                 135                 140

CGG GGG GCC TCT CAG CCG CCC ACC CCC ACC CCT GCC TCG GAT GCT TTT 480
Arg Gly Ala Ser Gln Pro Pro Thr Pro Thr Pro Ala Ser Asp Ala Phe
145                 150                 155                 160

CAG CGC AAG CTG GAG GGC TGC AGG TTC CTG CAT GGC TAC CAT CGC TTC 528
Gln Arg Lys Leu Glu Gly Cys Arg Phe Leu His Gly Tyr His Arg Phe
                165                 170                 175

ATG CAC TCA GTG GGG CGG GTC TTC AGC AAG TGG GGG GAG AGC CCG AAC 576
Met His Ser Val Gly Arg Val Phe Ser Lys Trp Gly Glu Ser Pro Asn
            180                 185                 190

CGG AGC CGG AGA TAA                                             591
Arg Ser Arg Arg ***
        195
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:603

(B) TYPE:nucleic acid

(C) STRANDEDNESS:double stranded

(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

```
ATG GCT TTC ACA GAG CAT TCA CCG CTG ACC CCT CAC CGT CGG GAC CTC  48
Met Ala Phe Thr Glu His Ser Pro Leu Thr Pro His Arg Arg Asp Leu
                5                   10                  15

TGT AGC CGC TCT ATC TGG CTA GCA AGG AAG ATT CGT TCA GAC CTG ACT  96
Cys Ser Arg Ser Ile Trp Leu Ala Arg Lys Ile Arg Ser Asp Leu Thr
            20                  25                  30

GCT CTT ACG GAA TCC TAT GTG AAG CAT CAG GGC CTG AAC AAG AAC ATC 144
Ala Leu Thr Glu Ser Tyr Val Lys His Gln Gly Leu Asn Lys Asn Ile
        35                  40                  45

AAC CTG GAC TCT GCG GAT GGG ATG CCA GTG GCA AGC ACT GAT CAG TGG 192
Asn Leu Asp Ser Ala Asp Gly Met Pro Val Ala Ser Thr Asp Gln Trp
    50                  55                  60

AGT GAG CTG ACC GAG GCA GAG CGA CTC CAA GAG AAC CTT CAA GCT TAT 240
Ser Glu Leu Thr Glu Ala Glu Arg Leu Gln Glu Asn Leu Gln Ala Tyr
65                  70                  75                  80

CGT ACC TTC CAT GTT TTG TTG GCC AGG CTC TTA GAA GAC CAG CAG GTG 288
Arg Thr Phe His Val Leu Leu Ala Arg Leu Leu Glu Asp Gln Gln Val
                85                  90                  95

CAT TTT ACC CCA ACC GAA GGT GAC TTC CAT CAA GCT ATA CAT ACC CTT 336
His Phe Thr Pro Thr Glu Gly Asp Phe His Gln Ala Ile His Thr Leu
            100                 105                 110

CTT CTC CAA GTC GCT GCC TTT GCA TAC CAG ATA GAG GAG TTA ATG ATA 384
Leu Leu Gln Val Ala Ala Phe Ala Tyr Gln Ile Glu Glu Leu Met Ile
        115                 120                 125
```

```
CTC CTG GAA TAC AAG ATC CCC CGC AAT GAG GCT GAT GGG ATG CCT ATT 432
Leu Leu Glu Tyr Lys Ile Pro Arg Asn Glu Ala Asp Gly Met Pro Ile
    130                 135                 140

AAT GTT GGA GAT GGT GGT CTC TTT GAG AAG AAG CTG TGG GGC CTA AAG 480
Asn Val Gly Asp Gly Gly Leu Phe Glu Lys Lys Leu Trp Gly Leu Lys
145                 150                 155                 160

GTG CTG CAG GAG CTT TCA CAG TGG ACA GTA AGG TCC ATC CAT GAC CTT 528
Val Leu Gln Glu Leu Ser Gln Trp Thr Val Arg Ser Ile His Asp Leu
                165                 170                 175

CGT TTC ATT TCT TCT CAT CAG ACT GGG ATC CCA GCA CGT GGG AGC CAT 576
Arg Phe Ile Ser Ser His Gln Thr Gly Ile Pro Ala Arg Gly Ser His
            180                 185                 190

TAT ATT GCT AAC AAC AAG AAA ATG TAG                             603
Tyr Ile Ala Asn Asn Lys Lys Met ***
        195                 200
```

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:537
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

```
CCT GGG CCA CCA CCT GGC CCC CCT CGA GTT TCC CCA GAC CCT CGG GCC  48
Pro Gly Pro Pro Pro Gly Pro Pro Arg Val Ser Pro Asp Pro Arg Ala
                  5                  10                  15

GAG CTG GAC AGC ACC GTG CTC CTG ACC CGC TCT CTC CTG GCG GAC ACG  96
Glu Leu Asp Ser Thr Val Leu Leu Thr Arg Ser Leu Leu Ala Asp Thr
            20                  25                  30

CGG CAG CTG GCT GCA CAG CTG AGG GAC AAA TTC CCA GCT GAC GGG GAC 144
Arg Gln Leu Ala Ala Gln Leu Arg Asp Lys Phe Pro Ala Asp Gly Asp
        35                  40                  45

CAC AAC CTG GAT TCC CTG CCC ACC CTG GCC ATG AGT GCG GGG GCA CTG 192
His Asn Leu Asp Ser Leu Pro Thr Leu Ala Met Ser Ala Gly Ala Leu
    50                  55                  60

GGA GCT CTA CAG CTC CCA GGT GTG CTG ACA AGG CTG CGA GCG GAC CTA 240
Gly Ala Leu Gln Leu Pro Gly Val Leu Thr Arg Leu Arg Ala Asp Leu
65                  70                  75                  80

CTG TCC TAC CTG CGG CAC GTG CAG TGG CTG CGC CGG GCA GGT GGC TCT 288
Leu Ser Tyr Leu Arg His Val Gln Trp Leu Arg Arg Ala Gly Gly Ser
                85                  90                  95

TCC CTG AAG ACC CTG GAG CCC GAG CTG GGC ACC CTG CAG GCC CGA CTG 336
Ser Leu Lys Thr Leu Glu Pro Glu Leu Gly Thr Leu Gln Ala Arg Leu
            100                 105                 110

GAC CGG CTG CTG CGC CGG CTG CAG CTC CTG ATG TCC CGC CTG GCC CTG 384
Asp Arg Leu Leu Arg Arg Leu Gln Leu Leu Met Ser Arg Leu Ala Leu
        115                 120                 125
```

```
CCC CAG CCA CCC CCG GAC CCG CCG GCG CCC CCG CTG GCG CCC CCC TCC 432
Pro Gln Pro Pro Pro Asp Pro Pro Ala Pro Pro Leu Ala Pro Pro Ser
    130                 135                 140

TCA GCC TGG GGG GGC ATC AGG GCC GCC CAC GCC ATC CTG GGG GGG CTG 480
Ser Ala Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu
145                 150                 155                 160

CAC CTG ACA CTT GAC TGG GCC GTG AGG GGA CTG CTG CTG CTG AAG ACT 528
His Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr
                165                 170                 175

CGG CTG TGA                                                     537
Arg Leu ***
        179
```

(2) INFORMATION FOR SEQ ID NO:7:
  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH:558
    (B) TYPE:nucleic acid
    (C) STRANDEDNESS:double stranded
    (D) TOPOLOGY:linear
  (xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

```
AAG CTT GTA CCT CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC  48
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
                5                   10                  15

AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC  96
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
            20                  25                  30

ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT 144
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
    35                  40                  45

AAC ATG TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC 192
Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
    50                  55                  60

CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT 240
Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65                  70                  75                  80

GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG 288
Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
                85                  90                  95

GTA TAC CTG GAA TAC CTG CAG AAC AGA TTT GAG AGC AGT GAG GAA CAA 336
Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
            100                 105                 110

GCC AGA GCT GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG 384
Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
        115                 120                 125

AAA AAG GCA AAG AAT CTA GAT GCA ATA ACC ACC CCT GAC CCC ACC CAG 432
Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Gln
    130                 135                 140
```

```
GGT GCC ATG CCG GCC TTC GCT AGC GCT TTC CAG CGC CGG GCA GGA GGG 480
Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
145                 150                 155                 160

GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC 528
Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
                165                 170                 175

GTT CTA CGC CAC CTT GCC CAG CCC TAG GAT                         558
Val Leu Arg His Leu Ala Gln Pro *** Asp
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:588
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

```
AAG CTT CCT CTG CCC ATC ACC CCT GTC AAC GCC ACC TGT GCC ATA CGC  48
Lys Leu Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg
                5                   10                  15

CAC CCA TGT CAC AAC AAC CTC ATG AAC CAG ATC AGG AGC CAA CTG GCA  96
His Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala
            20                  25                  30

CAG CTC AAT GGC AGT GCC AAT GCC CTC TTT ATT CTC TAT TAC ACA GCC 144
Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala
        35                  40                  45

CAG GGG GAG CCG TTC CCC AAC AAC CTG GAC AAG CTA TGT GGC CCC AAC 192
Gln Gly Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn
    50                  55                  60

GTG ACG GAC TTC CCG CCC TTC CAC GCC AAC GGC ACG GAG AAG GCC AAG 240
Val Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys
65                  70                  75                  80

CTG GTG GAG CTG TAC CGC ATA GTC GTG TAC CTT GGC ACC TCC CTG GGC 288
Leu Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly
                85                  90                  95

AAC ATC ACC CGG GAC CAG AAG ATC CTC AAC CCC AGT GCC CTC AGC CTC 336
Asn Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu
            100                 105                 110

CAC AGC AAG CTC AAC GCC ACC GCC GAC ATC CTG CGA GGC CTC CTT AGC 384
His Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser
        115                 120                 125

AAC GTG CTG TGC CGC CTG TGC AGC AAG TAC CAC GTG GGC CAT GTG GAC 432
Asn Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp
    130                 135                 140

GTG ACC TAC GGT CCG GAC CCA ACC ACA AAT GCC AGC CTG CTG ACG AAG 480
Val Thr Tyr Gly Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys
145                 150                 155                 160

CTG CAG GCA CAG AAC CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC ATT 528
Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile
                165                 170                 175
```

CTG CGC AGC TTT AAG GAG TTC CTG CAG TCC AGC CTG AGG GCT CTT CGG 576
Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg
180 185 190

CAA ATG TAG GAT
Gln Met *** Asp 588
195

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:582
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

AAG CTT CCT CTG CCC ATC ACC CCT GTC AAC GCC ACC TGT GCC ATA CGC 48
Lys Leu Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg
5 10 15

CAC CCA TGT CAC AAC AAC CTC ATG AAC CAG ATC AGG AGC CAA CTG GCA 96
His Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala
20 25 30

CAG CTC AAT GGC AGT GCC AAT GCC CTC TTT ATT CTC TAT TAC ACA GCC 144
Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala
35 40 45

CAG GGG GAG CCG TTC CCC AAC AAC CTG GAC AAG CTA TGT GGC CCC AAC 192
Gln Gly Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn
50 55 60

GTG ACG GAC TTC CCG CCC TTC CAC GCC AAC GGC ACG GAG AAG GCC AAG 240
Val Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys
65 70 75 80

CTG GTG GAG CTG TAC CGC ATA GTC GTG TAC CTT GGC ACC TCC CTG GGC 288
Leu Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly
85 90 95

AAC ATC ACC CGG GAC CAG AAG ATC CTC AAC CCC AGT GCC CTC AGC CTC 336
Asn Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu
100 105 110

CAC AGC AAG CTC AAC GCC ACC GCC GAC ATC CTG CGA GGC CTC CTT AGC 384
His Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser
115 120 125

AAC GTG CTG TGC CGC CTG TGC AGC AAG TAC CAC GTG GGC CAT GTG GAC 432
Asn Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp
130 135 140

GTG ACC TAC GGT CCG GAC ACA AAT GCC AGC CTG CTG ACG AAG CTG CAG 480
Val Thr Tyr Gly Pro Asp Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln
145 150 155 160

GCA CAG AAC CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC ATT CTG CGC 528
Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile Leu Arg
165 170 175

AGC TTT AAG GAG TTC CTG CAG TCC AGC CTG AGG GCT CTT CGG CAA ATG 576
Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln Met
180 185 190

TAG GAT 582
*** Asp
194

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:528
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

AAG CTT GTA CCT CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC 48
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC 96
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT 144
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 40 45

AAC ATG TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC 192
Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT 240
Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG 288
Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
85 90 95

GTA TAC CTA GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA 336
Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
100 105 110

GCC AGA GCT GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG 384
Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

AAA AAG GCA AAG AAT CTA GAT GCA ATA ACC ACC CCT GAT CCG GAC ACC 432
Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Asp Thr
130 135 140

TCG GGT AAG GAT GTC TTC CAG AAG AAG AAG CTG GGC TGT CAA CTC CTG 480
Ser Gly Lys Asp Val Phe Gln Lys Lys Lys Leu Gly Cys Gln Leu Leu
145 150 155 160

GGG AAG TAT AAG CAG ATC ATC GCC GTG TTG GCC CAG GCC TTC TAG GAT 528
Gly Lys Tyr Lys Gln Ile Ile Ala Val Leu Ala Gln Ala Phe *** Asp
165 170 175

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:522
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

```
AAG CTT GTA CCT CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC   48
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
                 5                  10                  15

AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC   96
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
             20                  25                  30

ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT  144
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
         35                  40                  45

AAC ATG TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC  192
Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
     50                  55                  60

CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT  240
Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
 65                  70                  75                  80

GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG  288
Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
                 85                  90                  95

GTA TAC CTA GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA  336
Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
            100                 105                 110

GCC AGA GCT GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG  384
Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
        115                 120                 125

AAA AAG GCA AAG AAT CTA GAT GCA ATA ACC ACT CCG GAC ACC TCG GGT  432
Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Thr Ser Gly
    130                 135                 140

AAG GAT GTC TTC CAG AAG AAG AAG CTG GGC TGT CAA CTC CTG GGG AAG  480
Lys Asp Val Phe Gln Lys Lys Lys Leu Gly Cys Gln Leu Leu Gly Lys
145                 150                 155                 160

TAT AAG CAG ATC ATC GCC GTG TTG GCC CAG GCC TTC TAG GAT          522
Tyr Lys Gln Ile Ile Ala Val Leu Ala Gln Ala Phe *** Asp
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:558
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
[illegible]
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

```
AAG CTT GTA CCT CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC   48
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
                 5                  10                  15

AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC   96
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
             20                  25                  30

ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT  144
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
         35                  40                  45
```

AAC ATG TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC 192
Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT 240
Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG 288
Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
85 90 95

GTA TAC CTA GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA 336
Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
100 105 110

GCC AGA GCT GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG 384
Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

AAA AAG GCA AAG AAT CTA GAT GCA ATA ACC ACC CCT GAT CCG GAC CAG 432
Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Asp Gln
130 135 140

GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA GGG 480
Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
145 150 155 160

GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC 528
Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
165 170 175

GTT CTA CGC CAC CTT GCC CAG CCC TAG GAT 558
Val Leu Arg His Leu Ala Gln Pro *** Asp
180 185

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:555
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

AAG CTT GTA CCT CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC 48
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC 96
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

ATC CTC GAC GGC ATC TCA GCC CTC CGG AAG GAG ACA TGT GCC ACC TAC 144
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Ala Thr Tyr
35 40 45

AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT CTG GGC 192
Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly
50 55 60

ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC AGC CAG GCC CTG CAG CTG 240
Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu
65 70 75 80

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GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG 288
Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly
                 85                  90                  95

CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC GAG TTG GGT CCC ACC TTG 336
Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu
            100                 105                 110

GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG 384
Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln
        115                 120                 125

CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC CTG CAA CCG GAC ACA AAT 432
Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Asp Thr Asn
    130                 135                 140

GCC AGC CTG CTG ACG AAG CTG CAG GCA CAG AAC CAG TGG CTG CAG GAC 480
Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp
145                 150                 155                 160

ATG ACA ACT CAT CTC ATT CTG CGC AGC TTT AAG GAG TTC CTG CAG TCC 528
Met Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser
                165                 170                 175

AGC CTG AGG GCT CTT CGG CAA ATG TAG                             555
Ser Leu Arg Ala Leu Arg Gln Met ***
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:582
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

```
AAG CTT CCT CTG CCC ATC ACC CCT GTC AAC GCC ACC TGT GCC ATA CGC  48
Lys Leu Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg
                 5                  10                  15

CAC CCA TGT CAC AAC AAC CTC ATG AAC CAG ATC AGG AGC CAA CTG GCA  96
His Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala
            20                  25                  30

CAG CTC AAT GGC AGT GCC AAT GCC CTC TTT ATT CTC TAT TAC ACA GCC 144
Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala
        35                  40                  45

CAG GGG GAG CCG TTC CCC AAC AAC CTG GAC AAG CTA TGT GGC CCC AAC 192
Gln Gly Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn
    50                  55                  60

GTG ACG GAC TTC CCG CCC TTC CAC GCC AAC GGC ACG GAG AAG GCC AAG 240
Val Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys
65                  70                  75                  80

CTG GTG GAG CTG TAC CGC ATA GTC GTG TAC CTT GGC ACC TCC CTG GGC 288
Leu Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly
                 85                  90                  95

AAC ATC ACC CGG GAC CAG AAG ATC CTC AAC CCC AGT GCC CTC AGC CTC 336
Asn Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu
            100                 105                 110
```

CAC AGC AAG CTC AAC GCC ACC GCC GAC ATC CTG CGA GGC CTC CTT AGC 384
His Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser
115 120 125

AAC GTG CTG TGC CGC CTG TGC AGC AAG TAC CAC GTG GGC CAT GTG GAC 432
Asn Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp
130 135 140

GTG ACC TAC GGT CCG GCG CCC CCG CTG GCG CCC CCC TCC TCA GCC TGG 480
Val Thr Tyr Gly Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser Ala Trp
145 150 155 160

GGG GGC ATC AGG GCC GCC CAC GCC ATC CTG GGG GGG CTG CAC CTG ACA 528
Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His Leu Thr
165 170 175

CTT GAC TGG GCC GTG AGG GGA CTG CTG CTG CTG AAG ACT CGG CTG TGA 576
Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg Leu ***
180 185 190

GGA TCC 582
Gly Ser
194

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:549
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

AAG CTT GTA CCT CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA CAC 48
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC 96
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT 144
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 40 45

AAC ATG TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC 192
Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT 240
Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

GTA TAC CTA GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA 288
Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
85 90 95

GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT GAG 336
Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
100 105 110

GCC AGA GCT GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG 384
Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

```
AAA AAG GCA AAG AAT CTA GAT GCA ATA ACC ACT CCG GCG CCC CCG CTG 432
Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Ala Pro Pro Leu
    130                 135                 140

GCG CCC CCC TCC TCA GCC TGG GGG GGC ATC AGG GCC GCC CAC GCC ATC 480
Ala Pro Pro Ser Ser Ala Trp Gly Gly Ile Arg Ala Ala His Ala Ile
145                 150                 155                 160

CTG GGG GGG CTG CAC CTG ACA CTT GAC TGG GCC GTG AGG GGA CTG CTG 528
Leu Gly Gly Leu His Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu
                165                 170                 175

CTG CTG AAG ACT CGG CTG TGA                                     549
Leu Leu Lys Thr Arg Leu ***
            180
```

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:537
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double stranded
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:16:

```
AAG CTT ACT CCT CTG GGC CCT GCC AGC TCC CTG CCC CAG AGC TTC CTG  48
Lys Leu Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu
                5                   10                  15

CTC AAG TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG  96
Leu Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala
            20                  25                  30

CTC CAG GAG AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG 144
Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu
        35                  40                  45

CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG AGC 192
Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser
    50                  55                  60

AGC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC CAA CTC 240
Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu
65                  70                  75                  80

CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG 288
His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly
                85                  90                  95

ATC TCC CCC GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC 336
Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val
            100                 105                 110

GCC GAC TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG 384
Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met
        115                 120                 125

GCC CCT GCC CTG CAA CCG GCG CCC CCG CTG GCG CCC CCC TCC TCA GCC 432
Ala Pro Ala Leu Gln Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser Ala
    130                 135                 140

TGG GGG GGC ATC AGG GCC GCC CAC GCC ATC CTG GGG GGG CTG CAC CTG 480
Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His Leu
145                 150                 155                 160
```

```
ACA CTT GAC TGG GCC GTG AGG GGA CTG CTG CTG CTG AAG ACT CGG CTG 528
Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg Leu
                165                 170                 175

TGA GGA TCC 537
*** Gly Ser
        179
```

(2) INFORMATION FOR SEQ ID NO:17:
  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 184
    (B) TYPE: amino acid
    (D) TOPOLOGY:linear
  (xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

```
Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
                5                   10                  15

Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
            20                  25                  30

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
        35                  40                  45

Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
    50                  55                  60

Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65                  70                  75                  80

Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
                85                  90                  95

Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
            100                 105                 110

Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
        115                 120                 125

Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Gln
    130                 135                 140

Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
145                 150                 155                 160

Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
                165                 170                 175

Val Leu Arg His Leu Ala Gln Pro
            180
```

(2) INFORMATION FOR SEQ ID NO:18:
  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 194
    (B) TYPE: amino acid
    (D) TOPOLOGY:linear
  (xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

```
Lys Leu Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg
                5                   10                  15

His Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala
            20                  25                  30
```

Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala
35 40 45

Gln Gly Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn
50 55 60

Val Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys
65 70 75 80

Leu Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly
85 90 95

Asn Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu
100 105 110

His Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser
115 120 125

Asn Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp
130 135 140

Val Thr Tyr Gly Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys
145 150 155 160

Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile
165 170 175

Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg
180 185 190

Gln Met

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 192
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:19:

Lys Leu Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg
5 10 15

His Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala
20 25 30

Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala
35 40 45

[illegible]
50 55 60

Val Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys
65 70 75 80

Leu Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly
85 90 95

Asn Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu
100 105 110

His Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser
115 120 125

Asn Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp
130 135 140

Val Thr Tyr Gly Pro Asp Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln
145 150 155 160

Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile Leu Arg
165 170 175

Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln Met
180 185 190

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 174
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 40 45

Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
85 90 95

Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
100 105 110

Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Asp Thr
130 135 140

Ser Gly Lys Asp Val Phe Gln Lys Lys Lys Leu Gly Cys Gln Leu Leu
145 150 155 160

Gly Lys Tyr Lys Gln Ile Ile Ala Val Leu Ala Gln Ala Phe
165 170

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 40 45

Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
85 90 95

Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
100 105 110

Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Thr Ser Gly
130 135 140

Lys Asp Val Phe Gln Lys Lys Lys Leu Gly Cys Gln Leu Leu Gly Lys
145 150 155 160

Tyr Lys Gln Ile Ile Ala Val Leu Ala Gln Ala Phe
165 170

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 184
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:22:

Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 40 45

Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
85 90 95

Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
100 105 110

Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Asp Gln
130 135 140

Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
145 150 155 160

Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
165 170 175

Val Leu Arg His Leu Ala Gln Pro
180

(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 184
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Ala Thr Tyr
35 40 45

Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly
50 55 60

Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu
65 70 75 80

Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly
85 90 95

Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu
100 105 110

Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln
115 120 125

Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Asp Thr Asn
130 135 140

Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp
145 150 155 160

Met Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser
165 170 175

Ser Leu Arg Ala Leu Arg Gln Met
180

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

Lys Leu Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg
5 10 15

His Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala
20 25 30

Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala
35 40 45

Gln Gly Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn
50 55 60

Val Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys
65 70 75 80

Leu Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly
85 90 95

Asn Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu
100 105 110

His Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser
115 120 125

Asn Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp
130 135 140

Val Thr Tyr Gly Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser Ala Trp
145 150 155 160

Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His Leu Thr
165 170 175

Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg Leu
180 185 190

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:25:

Lys Leu Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
5 10 15

Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 40 45

Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60

Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80

Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
85 90 95

Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
100 105 110

Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125

Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Ala Pro Pro Leu
130 135 140

Ala Pro Pro Ser Ser Ala Trp Gly Gly Ile Arg Ala Ala His Ala Ile
145 150 155 160

Leu Gly Gly Leu His Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu
165 170 175

Leu Leu Lys Thr Arg Leu
180

(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 176
(B) TYPE: amino acid
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

Lys Leu Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu
5 10 15

Leu Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala
20 25 30

Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu
35 40 45

Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser
50 55 60

Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu
65 70 75 80

His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly
85 90 95

Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val
100 105 110

Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met
115 120 125

Ala Pro Ala Leu Gln Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser Ala
130 135 140

Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His Leu
145 150 155 160

Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg Leu
165 170 175

What is Claimed is:

1. A hybrid cytokine polypeptide comprising: 1) three or four α-helical sequences, the α-helical sequences selected from cytokine α-helical sequences, the cytokine being selected from the group consisting of leukemia inhibitory factor (LIF), granulocyte colony stimulating factor (G-CSF), interleukin-6 (IL-6), interleukin-11 (IL-11), ciliary neurotrophic factor (CNTF), and oncostatin-M (OSM); and 2) at least three linking sequences, the linking sequences selected from at least a portion of a linking sequence from any of the foregoing cytokines, wherein at least one of the three or four α-helical sequences is derived from a different cytokine than at least one other of the three or four α-helical sequences and the hybrid cytokine further comprises encoded DNA sequences that hybridize under high stringency conditions to a probe derived from a DNA sequence encoding any of the foregoing hybrid cytokines.

2. A DNA molecule that encodes a hybrid cytokine, the hybrid cytokine comprising: 1) three or four α-helical sequences selected from an α-helical sequence derived from a cytokine, the cytokine being selected from the group consisting of LIF, G-CSF, IL-6, IL-11, CNTF and OSM; and 2) at least three linking sequences selected from at least a portion of a linking sequence from any of the foregoing cytokines, wherein at least one of the three or four α-helical sequences is from a different cytokine than at least one other of the three or four α-helical sequences, said DNA molecule comprising:

(A) complementary strands;

(B) DNA molecules which hybridize, under conditions of high stringency, to a probe consisting of any of the foregoing DNA molecules or their complementary sequences; and

(C) DNA molecules which would hybridize to the DNA molecules set forth above, but for a degeneracy of genetic code.

3. The polypeptide or DNA molecule according to claim 1 or 2, comprising four α-helical sequences.

4. The polypeptide or DNA molecule according to claim 1or 2, wherein each of the three or four α-helical sequences comprises a corresponding α-helical sequence of any of the cytokines.

5. The polypeptide or DNA molecule according to claim 1 or 2, wherein hybrid α-helical sequences I, II and III comprise α-helical sequences from the same cytokine and hybrid α-helical sequence IV comprises an α-helical sequence from a different cytokine.

6. The polypeptide or DNA molecule according to claim 1 or 2, wherein hybrid α-helical sequences II, III and IV comprise α-helical sequences from the same cytokine and hybrid α-helical sequence I comprises an α-helical sequence from a different cytokine.

7. The polypeptide or DNA molecule according to claim 1 or 2, wherein hybrid α-helical sequences II and III comprise α-helical sequences from the same cytokine.

8. The polypeptide or DNA molecule according to claim 1 or 2, wherein hybrid α-helical sequences I and IV comprise α-helical sequences from the same cytokine.

9. The polypeptide or DNA molcculc according to claim 1 or 2, wherein hybrid cytokines maintain a relative polarity orientation of hybrid α-helical sequences I-IV corresponding to a natural polarity oricntation.

10. The polypeptide or DNA molecule according to claim 1 or 2, wherein hybrid α-helical sequences I and IV have anti-parallel orientation.

11. The polypeptide or DNA molecule according to claim 1 or 2, wherein the hybrid cytokine α-helical sequences are selected from the group consisting of: GGGI; OOOI; LLLI; IIIO; GGGO; OOOG; LLLO; IIIG; GGGL; OOOL; LLLG; IIIL; IGGG; IOOO; ILLL; OIII; OGGG; GOOO; OLLL; GIII; LGGG; LOOO; GLLL; LIII; GLLG; GIIG; IGGI; LOGI; LLII; LLGG; IIGG; EGGG; OOOE; LLLE; IIIE; LEEE; CEEE; ECCC; EEEC; GCCC; CCCE; LLLC; OOOC; IIIC; GCCG; CGGC; LCCC; CCLL; CCII; CGGG; CELI; and ECCE.

12. The polypeptide or DNA molecule according to claim 1 or 2, wherein the hybrid cytokine α-helical sequences are selected from the group consisting of: GGLL; GGII; GGOO; GGGI; IGGG; GILO; LOGI; LLII; GGGO; GGGL; OOOG; LLLG; GOOO; OGGG; LGGG and GGGI.

13. A biologically functional plasmid or viral DNA vector comprising the molecule according to claim 2.

14. An expression system for expressing the hybrid cytokine comprising the molecule of claim 2, operably linked to control sequences and compatible with a recombinant host stably transfected with the molecule.

15. Recombinant host cells transformed with the expression system of claim 14.

16. A method for expessing a hybrid cytokinc of claim 1, comprising the steps of:

culturing recombinant host cells transformed with an expression system for expressing the hybrid cytokine comprising the molecule of claim 1, said DNA operably linked to control sequences and compatible with the recombinant host cells, under conditions which effect expression of said hybrid cytokine, and

recovering said hybrid cytokine.

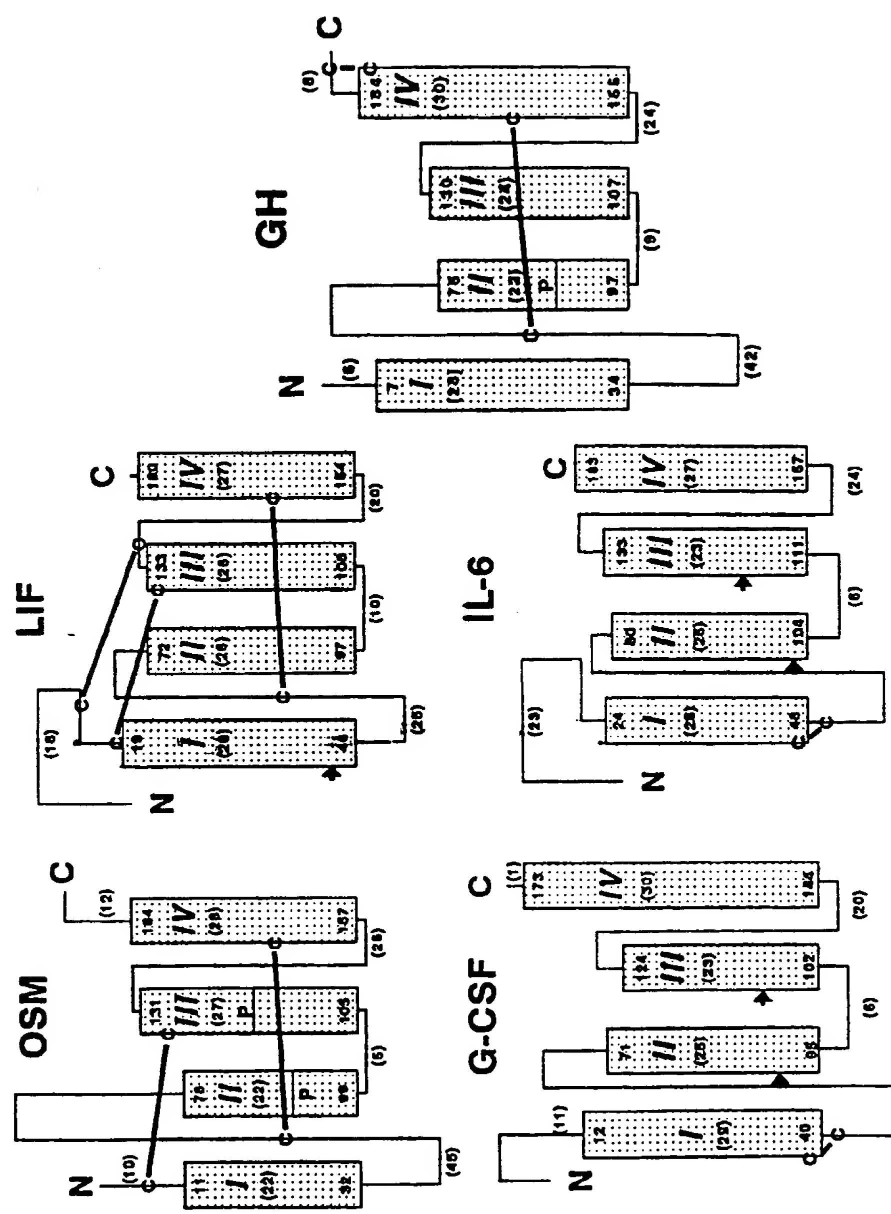

FIGURE 1

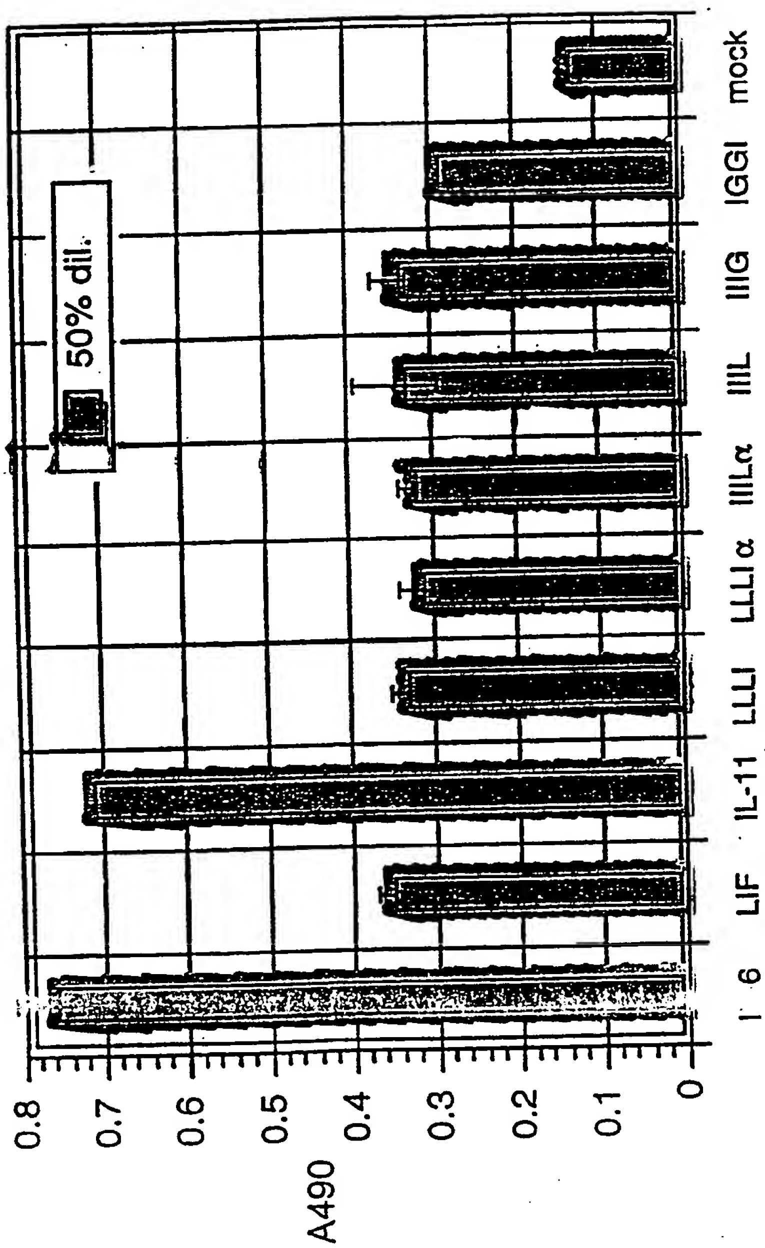
A490
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
50% dil.
I 6
LIF
IL-11
LLLI
LLLI α
IIILα
IIIL
IIIG
IGGI
mock

FIGURE 2

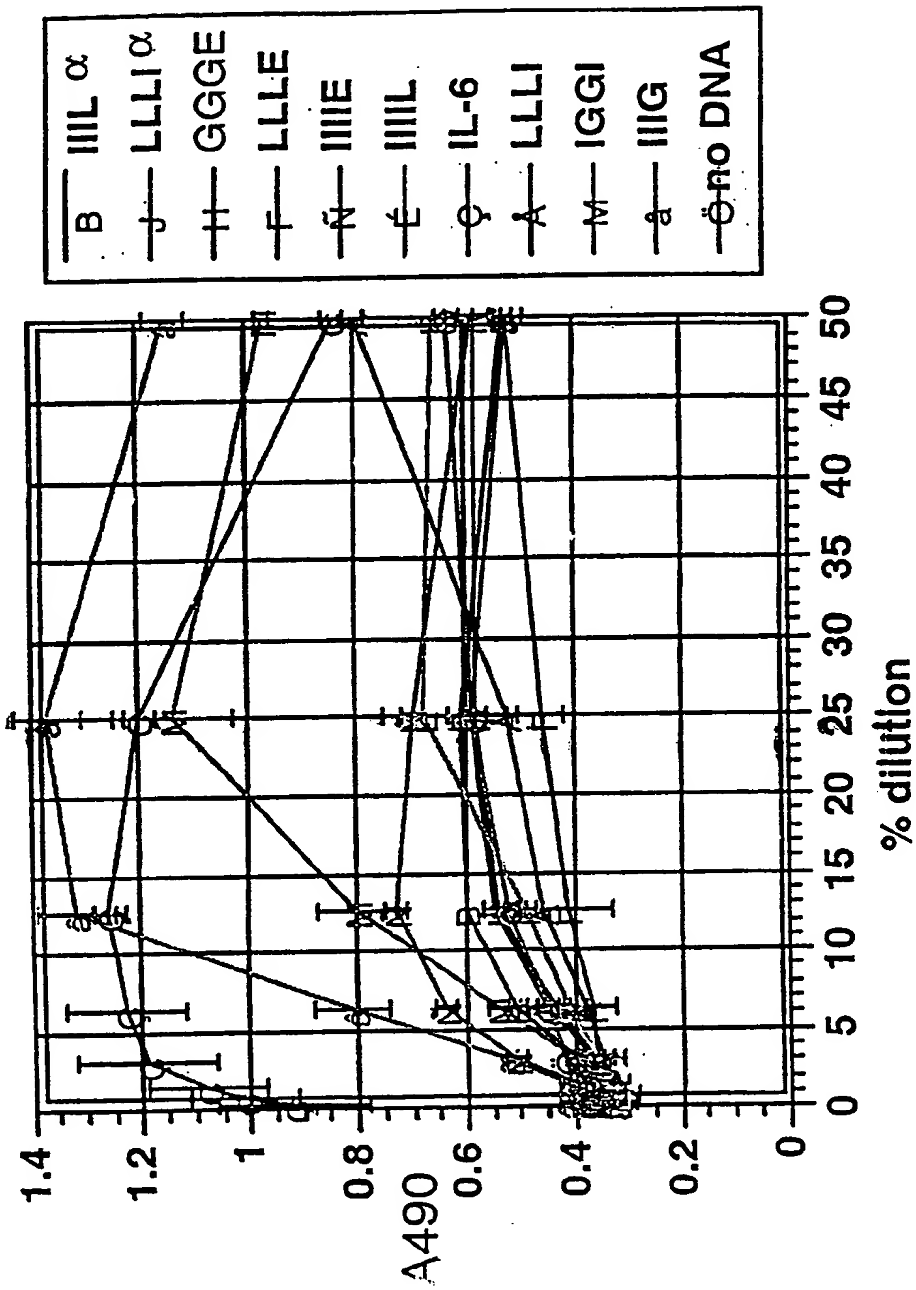
IIIL α
LLLI α
GGGE
LLLE
IIIIE
IIIIL
IL-6
LLLI
IGGI
IIIG
no DNA
A490
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
40
45
50
% dilution

FIGURE 4

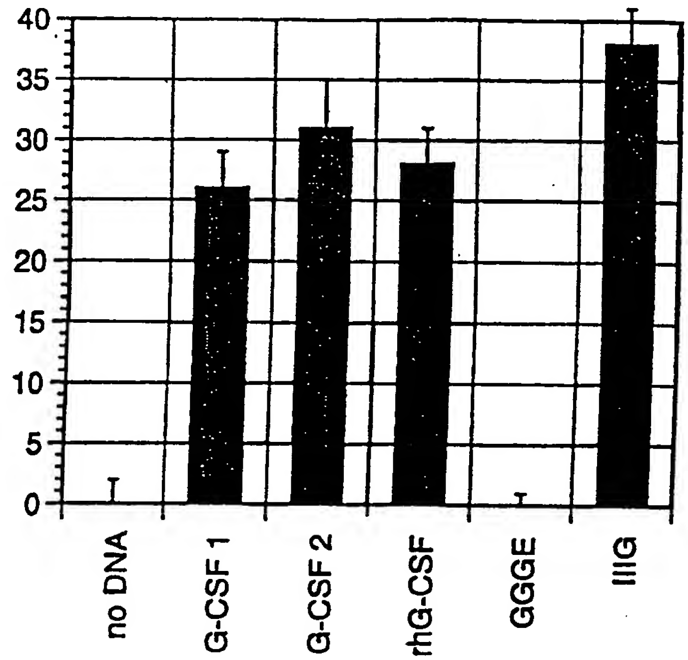

FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12873

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12P 21/06; C12N 5/00, 15/00; C07K 14/00; C07H 17/00
US CL :435/69.52, 69.7, 240.1, 320.1; 536/23.4, 23.51; 530/351
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.52, 69.7, 240.1, 320.1; 536/23.4, 23.51; 530/351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Intelligenetics

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Neuroscience Research, Vol. 15, issued 1992, T. Yamamori et al., "Coevolution of cytokine receptor families in the immune and nervous systems", pages 151-161.	1-16
A	Protein Engineering, Vol. 5, No. 6, issued 06 September 1992, F. Rock et al., "Overexpression and structure-function analysis of bioengineered IL-2/IL-6 chimeric lymphokine" pages 583-591.	1-16
A	Cancer, Vol. 67, issued 1991, D.E. Williams et al., "Hematopoietic effects of granulocyte-macrophage colony-stimulating factor/interleukin-3 fusion protein", pages 2705-2707.	1-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 JANUARY 1995	03 MAR 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Karen Cochrane Carlson, Ph.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBO J., Vol. 10, No. 13, issued 1991, A.B. Shanafelt et al., "The amino-terminal helix of GM-CSF and IL-5 governs high affinity binding to their receptors", pages 4105-4112.	1-16
A	WO 90/12877 (RALPH ET AL) 01 November 1990, see entire document.	1-16
A	US, A, 4,935,233 (BELL ET AL) 19 June 1990, see entire document.	1-16
A,P	Science, Vol. 263, issued 07 January 1994, N. Stahl et al., "Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 B receptor components", pages 92-95, see entire document.	1-16
A	Proc. Natl. Acad. Sci., Vol. 88, issued October 1991, T.M. Rose et al., "Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6", pages 8641-8645, see entire document.	1-16
A,P	US, A, 5,371,193 (BENNETT ET AL) 06 December 1994, see entire document.	1-16